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A CASE OF YOLK FORMATION NOT CONNECTED WITH THE PRODUCTION OF OVA.

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The secretion or production of true yolk in situations other than in ova or in the follicular cells which surround ova is not known so far as I am aware. "Nurse" or "yolk" cells have of course long been known to exist in several groups of animals; these, however, are evidently the equivalents of follicular cells or of ova. It is therefore of some interest to record the finding of true yolk in spaces within the connective tissues which lie externally to the follicular membrane of capsules which had previously liberated ova.

These observations were made on the ovaries of the common fowl during the mid-summer season. During July and August of the present summer the writer had occasion to examine the ovaries of more than one hundred full-grown hens. Among these there were at least six or eight ovaries which showed unmistakably the peculiar accumulation and placement of yolk which is here described.

In order to be sure that one is really dealing with "extra-ovular" and "extra-follicular" formation of yolk, and not merely with a masque of its usual source, it was necessary to determine three things concerning the capsules within which the yolk in question was found: (1) That an egg had been surely developed and liberated from this capsule. (2) That the space in which the yolk was found is quite separate and removed from the space formerly occupied by the ovum, and likewise removed from the follicular cells which surrounded the ovum. (3) That the accumulated substance is true yolk. I believe that the material I have examined has enabled me satisfactorily to determine each of these points.

The evidence that the yolk-containing capsules in question had previously borne and liberated ova rests partially upon the finding of capsules showing all the intermediate stages between

the recently broken capsules and the large, flabby, often asymmetrical, yolk-containing ones. Some of these latter capsules might be mistaken for resorbed ova, since they too have a closed stigma; that is, the slit or splitting which occurs in the capsule at the time of ovulation, and which allows the escape of the ovum, later heals together and the cavity of the follicle is once more completely sealed. The chances for such confusion are further increased by the fact that this central chamber may also occasionally re-accumulate yolk.

It is possible nevertheless in favorable material to be quite sure that the stigma has been *broken* and reunited—a thickened, accentuated, and often more or less ragged point of reunion indicating this. Furthermore, a series of follicles in the *same* ovary, showing the most recent ones still broken open, often decides the matter at once with certainty. The capsule from which a sample of yolk for analysis was taken was one of such a series. In this case there were nine yolk-containing capsules in various stages of extra-ovular yolk-production; and in addition, one other—the newest follicle—plainly recognized by its whole appearance as a recently emptied one. This follicle, however, showed the once *broken lips of the stigma now nearly completely grown together*, but with its inner cavity as *clean and free from yolk* as at the moment of ovulation. It is certain that the follicles of this ovary had liberated ova, and that instead of degenerating thereafter these capsules quickly closed the breaches formed in extruding the ova, and began the production of yolk in their external walls.

It is easy to demonstrate that the yolk-filled spaces bulging from the sides of the capsules have no open connection with the central cavity of the capsule; that is to say, these spaces are not connected with the former seat of yolk formation. Several times I have made a slit in the scar or stigma and, finding the interior clean and free from yolk, have tried by squeezing the various bags of yolk lying in the external walls of the capsule to make their yolk flow into the central cavity. In no instance have I succeeded in thus finding any connection whatever between these new yolk-containing cavities and the old cavity formerly occupied by the egg. On the contrary, careful dissections of

these capsules show that the two spaces are always separated by a rather thick wall; certainly much thicker than that which separates the new yolk space from the exterior. This latter wall, in fact, is usually very thin. It consists, however, of an extremely thin connective tissue layer in addition to the ovarian epithelium. By careful handling the epithelium can be stripped off and the thin layer enclosing the yolk space left intact.

The very external position of the yolk spaces—of which there may be several in a single capsule—makes it evident that none of the cells of the old follicular membrane are engaged in the production of yolk in this new and unusual site. The production of this yolk is necessarily accomplished by the cells which form the external theca—a tissue from the former ovarian stroma, which in the late growth stages of the capsule of large eggs becomes a very thick, firm, essentially connective tissue layer enclosing possibly some scattered derivatives of the germinal epithelium—whose cells normally take no part in yolk formation.

Just what it is that transforms these non-yolk-producing cells into cells actively engaged in yolk production, it would be most interesting to know. While confessing very complete ignorance as to this cause, it seems worth while to note that *the cells which here take up a new function do so at the time when the "normal" thing for them to do is to degenerate and be absorbed.*

In this connection it should be stated that the true follicular cells—those which have previously been engaged in passing on the constituents of yolk to the egg—are apparently the least liable of any of the capsular cells to take part in any later yolk production. Only occasionally in a group of capsules, each of which may be producing yolk at one or more points externally, will one find that the follicular cells have continued—or rather have recommenced—to produce yolk. What I have observed would indicate that these follicle cells never in any case become active until after yolk production has been initiated in the more external layers; but of this latter point I am not certain.

That the yellowish fluid enclosed in these yolk spaces is true yolk is indicated by its microscopic appearance. The question is positively and affirmatively answered by the chemical analysis

of a sample. 1.605 grams of such yolk were collected from a single one of the new yolk spaces; this was not all, but nearly all of the contents of the cavity. In order to show how closely its chemical composition agrees with that of other forms of true yolk, I have added to the table the numbers resulting from the analysis of four such samples of yolk. Reference to the table readily shows the essential similarity of all these substances; and likewise a point or two of notable difference.

Analysis of:	In Per. Cent. of Solids.					H ₂ O.
	Leci- thin.	Protein.	Neutral Fat.	Total Ash.	Organic Extractives.	
Extra-follicular yolk	19.05	26.21	45.39	6.61	2.65	74.22
Central "yolk body" from in- cubated hen's egg	19.68	28.87	46.05	3.40	2.00	37.13
Egg yolk, Jungle fowl	19.90	30.47	46.74	1.30	1.59	48.70
Contents of yolk-sac; 21 days in- cubation	17.62	33.24	47.36	1.16	1.39	56.52
Resorbed ovum	15.30	35.18	42.25	1.71	2.08	63.20

It is true that I have selected for this comparison analyses which most closely agree with the analysis of the "extra-follicular" yolk. The high water content of the latter is of no consequence; an analysis of "white" yolk from the hen having yielded more than 80.0 per cent. of this constituent.

The high ash content, and very low protein content, do indicate however a species of yolk not in all respects like that produced by the follicular cell and the ovum. In these two respects this yolk stands as a rather bold extreme in a long series of analyses of normal yolk. It can be said therefore that though this substance is certainly "yolk," its peculiar origin stamps its chemical composition with a specificity of its own.

The foregoing recital of the facts is perhaps hardly sufficient to uncover at once to every reader one of the points of interest in these findings; at any rate it is a point of interest to the writer. I refer to the fact that in all of the hitherto known cases of yolk formation the whole process of yolk building and storage appears so glaringly and profoundly teleological. The ovum prepares and stores food for an embryo that is yet to form; a follicular cell passes on this rich material only to an ovum which in turn accumulates for a promised organism that will arise and

require the store; ovum and ten thousand follicular cells unite to prepare and to hoard a pabulum for an organism whose father exists as yet only in prophecy and in fortune; a "nurse" cell arises in a distant part, migrates with its supplies and unerringly delivers all to the egg—whose prospective accomplishment only can use or require them; or, again, as in some hydroids, several adjacent ova laboriously produce a golden store which together with their own existence they place sacrificially upon the altar of posterity—giving all to a more opulent neighbor, who through the combined accumulations of many gatherers can the more adequately and assuredly provide for the beginning of an individual that is to be.

Nor is such apparent teleology absent from the very chemical composition of the material that is stored. The developing organism requires above all else a store and source of energy, and one notes that yolk—the material actually stored—is richer in lecithin and fat than is any other product of the body; and further that these constituents are the ones which carry far more energy per unit of weight or volume than do any others.

When, however, one turns to the sort of yolk formation described in this paper,—yolk formation which begins in subdued and atretic follicles, among cells largely "somatized" and doomed to certain degeneration; when one considers the utter blindness involved in these ill-conditioned cells plunging into a most active production of excessively rich foods, only to cast them into the formless spaces of these spent capsules, one can realize that the process of yolk building actually can be as grotesquely absurd and inappropriate as it has elsewhere seemed replete with insistent teleology.

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